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Figures and figure supplements

Methylation at the C-2 position of hopanoids increases rigidity in native bacterial membranes

Chia-Hung Wu, et al.

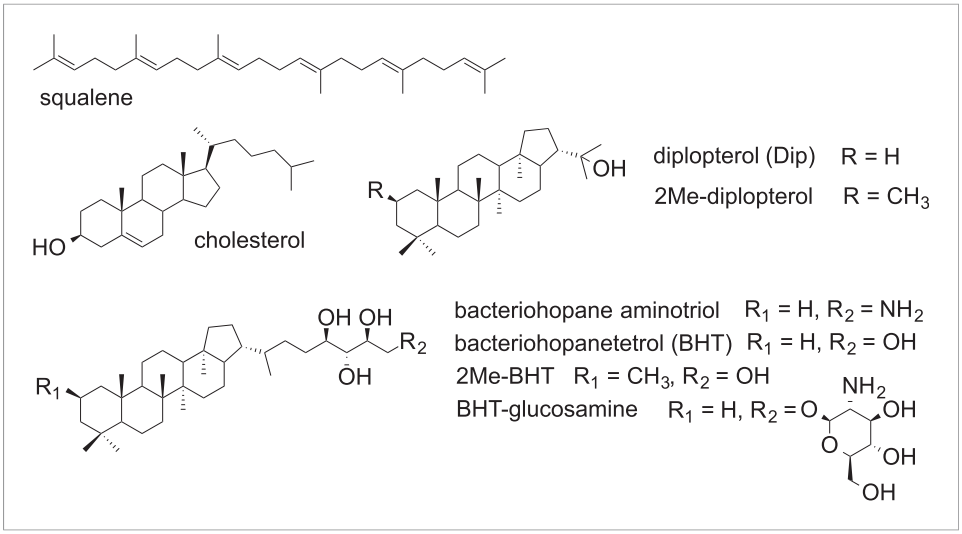

Figure 1. Structures of selected hopanoids, cholesterol, and squalene.
DOI: 10.7554/eLife.05663.003

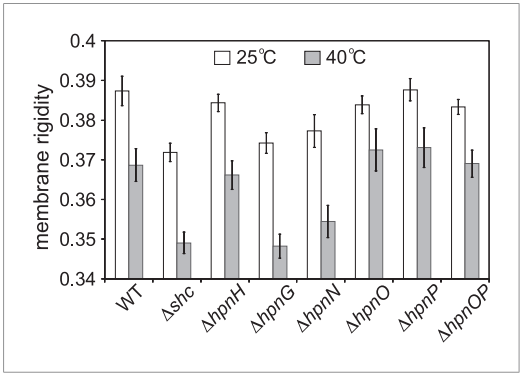

Figure 2. Whole cell membrane fluidity. Error bars represent the standard deviation from three biological replicates (total 21–26 technical replicates).
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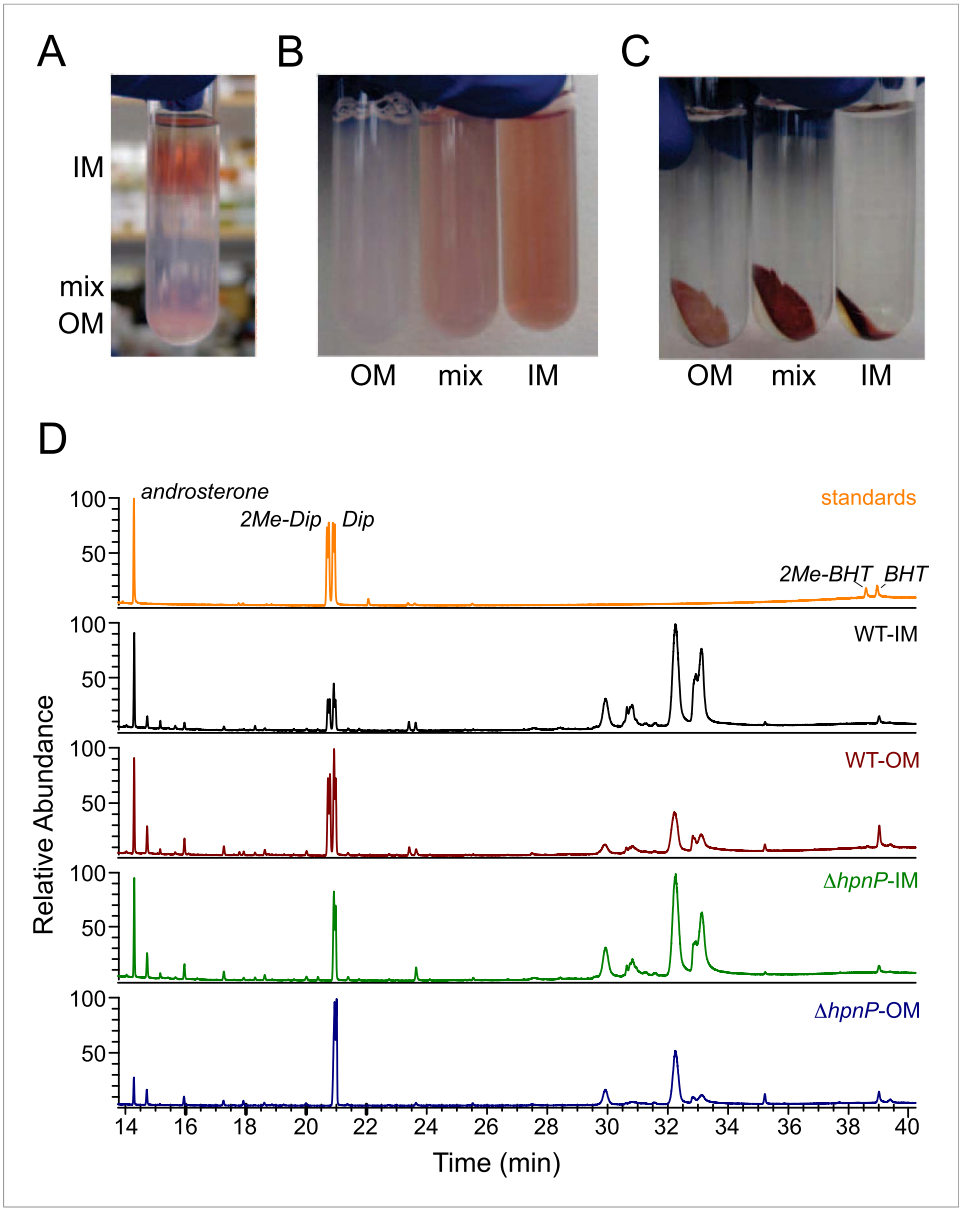

Figure 3. Membrane fractionation and hopanoids analysis using GC–MS. (**A**) Three distinct bands were formed after ultracentrifugation in a Percoll gradient. (**B**) The bands were recovered and resuspended. (**C**) Samples were ultracentrifuged to pellet down the purified membranes, which sat on top of a transparent solid Percoll layer. (**D**) GC–MS of fractionated membranes of *R. palustris* TIE-1 WT and Δ*hpnP*.

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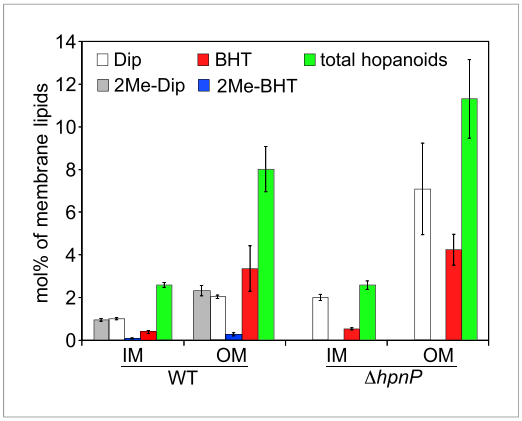

Figure 4. Molar percentage of hopanoids in the inner membrane (IM) and outer membrane (OM) of WT and Δ*hpnP* determined by GC–MS. Error bars represent the standard deviation from three biological replicates. Total hopanoids = sum of (2Me)-Dip and (2Me)-BHT.

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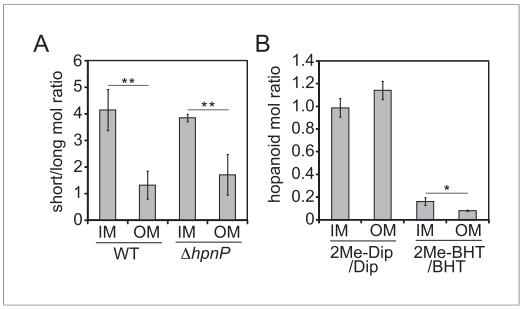

Figure 5. Partitioning of hopanoids in the inner membrane (IM) and outer mebraane (OM) of *R. palustris* TIE-1. (**A**) Molar ratio between short (Dip and 2Me-Dip) and long (BHT and 2Me-BHT) hopanoids in WT and Δ*hpnP*. (**B**) Molar ratio between methylated and desmethylated hopanoid in WT. Error bars represent the standard deviation from three biological replicates. *p = 0.015; **p < 0.01.

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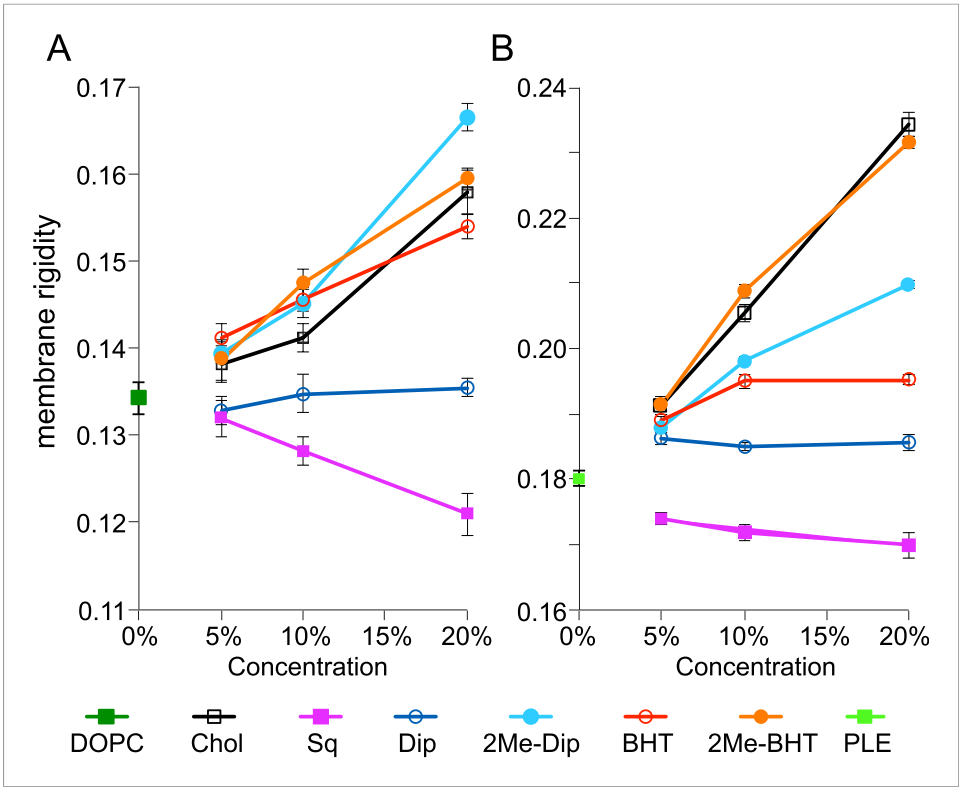

Figure 6. Membrane rigidity measurements at 25°C using model lipids. (**A**) Dioleoyl phosphatidylcholine (DOPC) and (**B**) *E. coli* polar lipid extract (PLE) were mixed with different mol% of cholesterol, squalene, and hopanoids. Error bars represent the standard deviation from three biological replicates (total 21 technical replicates).

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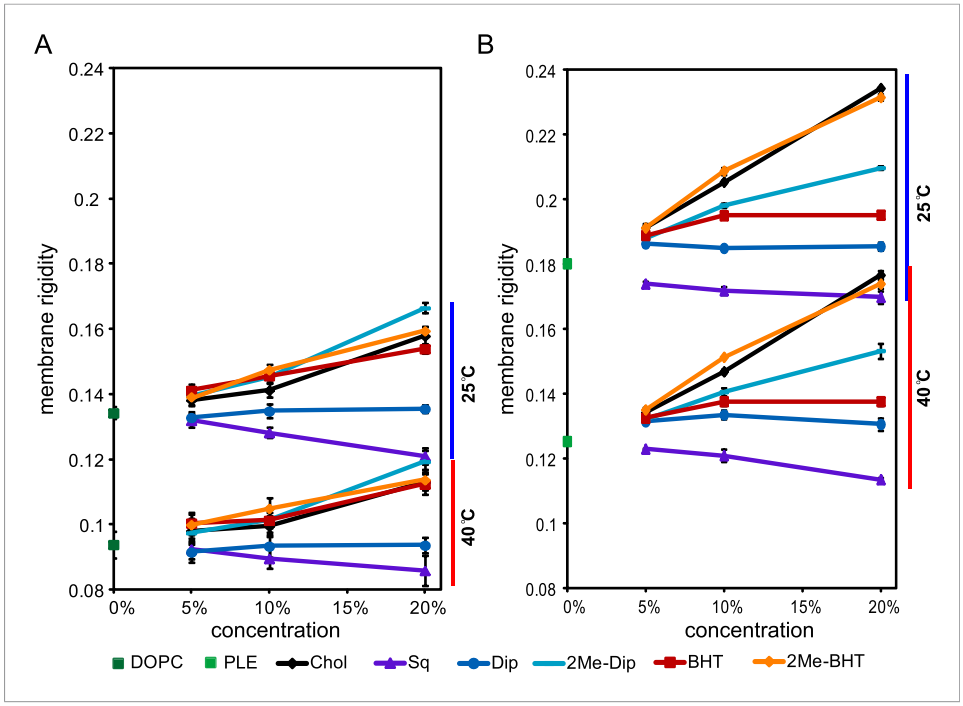

Figure 6—figure supplement 1. Membrane rigidity measurements at 25˚C and 40˚C using model lipids. (**A**) DOPC and (**B**) *E. coli* polar lipid extract (PLE) were mixed with different mol% of cholesterol, squalene, and hopanoids. Error bars represent standard deviation from three biological replicates (total 21 technical replicates).

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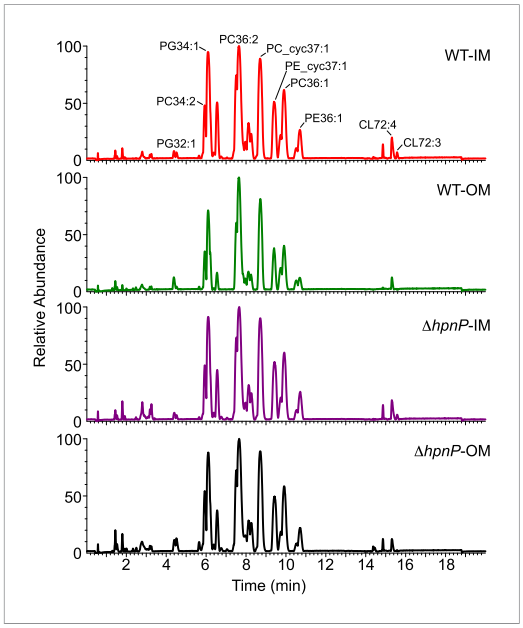

Figure 7. LC-MS profiles of the inner membrane (IM) and outer membrane (OM) of *R. palustris* TIE-1 WT and Δ*hpnP*.

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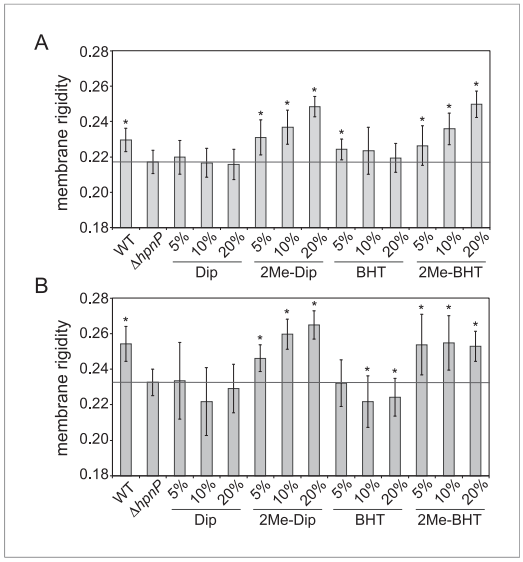

Figure 8. Membrane rigidity measurements at 25˚C using total lipid extract from *R. palustris* TIE-1 inner membrane (IM) and outer membrane (OM). The IM (**A**) or OM (**B**) from Δ*hpnP* was mixed with different mol% of hopanoids. Error bars represent the standard deviation from three biological replicates (total 21 technical replicates). *p < 0.001 (relative to Δ*hpnP*).

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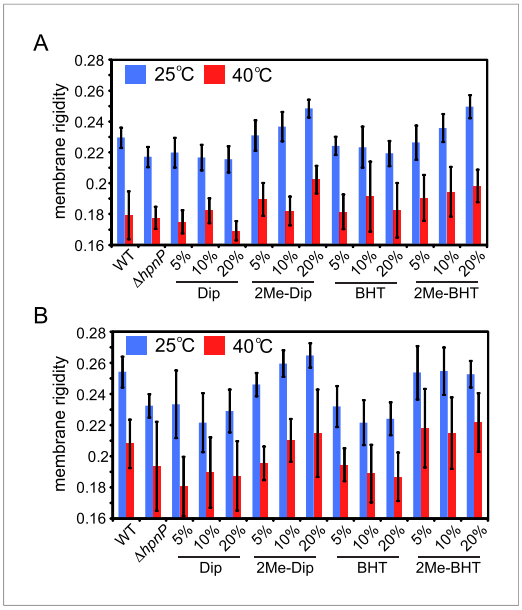

Figure 8—figure supplement 1. Membrane rigidity measurements at 40˚C using total lipid extract from *R. palustris* TIE-1 inner membrane (IM) and outer membrane (OM). The IM (**A**) or OM (**B**) from Δ*hpnP* was mixed with different mol% of hopanoids. Small unilamellar vesicles from the lipid mixtures were prepared, and the membrane rigidity was measured by fluorescence polarization of a reporter dye diphenyl hexatriene. Error bars represent standard deviation from three biological replicates (total 21 technical replicates).
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